

# Dietary Choline and Betaine Intakes Vary in an Adult Multiethnic Population<sup>1–3</sup>

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## Abstract

Choline and betaine are important nutrients for human health, but reference food composition databases for these nutrients became available only recently. We tested the feasibility of using these databases to estimate dietary choline and betaine intakes among ethnically diverse adults who participated in the Multiethnic Cohort (MEC) Study. Of the food items ( $n = 965$ ) used to quantify intakes for the MEC FFQ, 189 items were exactly matched with items in the USDA Database for the Choline Content of Common Foods for total choline, choline-containing compounds, and betaine, and 547 items were matched to the USDA National Nutrient Database for Standard Reference for total choline ( $n = 547$ ) and 148 for betaine. When a match was not found, choline and betaine values were imputed based on the same food with a different form (124 food items for choline, 300 for choline compounds, 236 for betaine), a similar food ( $n = 98, 284, \text{ and } 227$ , respectively) or the closest item in the same food category ( $n = 6, 191, \text{ and } 157$ , respectively), or the values were assumed to be zero ( $n = 1, 1, \text{ and } 8$ , respectively). The resulting mean intake estimates for choline and betaine among 188,147 MEC participants (aged 45–75) varied by sex (372 and 154 mg/d in men, 304 and 128 mg/d in women, respectively;  $P$ -heterogeneity  $< 0.0001$ ) and by race/ethnicity among Caucasians, African Americans, Japanese Americans, Latinos, and Native Hawaiians ( $P$ -heterogeneity  $< 0.0001$ ), largely due to the variation in energy intake. Our findings demonstrate the feasibility of assessing choline and betaine intake and characterize the variation in intake that exists in a multiethnic population. *J. Nutr.* 143: 894–899, 2013.

## Introduction

Choline and betaine are important nutrients for human development and health (1,2). Choline is an essential nutrient and a precursor to the neurotransmitter acetylcholine, to phospholipids that constitute cell membrane and transport cholesterol, and to betaine that provides up to 60% of the methyl moieties required for DNA methylation, synthesis, and repair (3,4). Betaine, consumed in foods or endogenously derived from choline, also serves as an osmolyte (2). Studies have indicated that dietary choline promotes perinatal development (5–9), and an insufficiency may increase adults' risk for hepatic steatosis (fatty liver) and other liver damage (10). In fact, the dietary recommendation established for choline [an Adequate Intake (AI) of 550 mg/d for men and 425 mg/d for women] is based on the choline requirement to prevent liver damage in healthy adult men (11,12). Although research on these nutrients is of high priority (12), they have been examined in only a few epidemiologic studies of

chronic diseases associated with aberrant DNA methylation and synthesis (13–15). This is likely because the reference food composition databases (FCDs)<sup>4</sup> for dietary assessment of these nutrients have become available only in recent years (16,17).

Choline is found in foods as free choline or as choline esters, including glycerophosphocholine, phosphocholine, phosphatidylcholine (lecithin), and sphingomyelin, with the reported main food sources being red meat, eggs, poultry, and milk (15,18). Betaine is obtained mostly from grain products and spinach (15,18). The food content information for total choline, choline-containing compounds (i.e., free choline and choline esters that sum up to total choline), and betaine is available from the USDA Database for the Choline Content of Common Foods, created in 2004 and updated in 2008 (16,17). The total choline and betaine contents in some additional food items are also found in the USDA National Nutrient Database for Standard Reference (19).

Limited epidemiologic studies on dietary intake of choline and betaine indicate that a substantial proportion of adults may have intake below the AI levels (9,13,20). Even fewer data are available for choline and betaine intake in non-Caucasian populations, whose dietary patterns are known to vary and whose

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<sup>3</sup> Supplemental Figures 1 and 2 and Supplemental Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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<sup>4</sup> Abbreviations used: AI, Adequate Intake; choline database, the USDA Database for Choline Content of Common Foods (Release Two, 2008); FCD, food composition database; MEC, Multiethnic Cohort; MSG, monosodium glutamate; SR, USDA National Nutrient Database for Standard Reference (Release 20, 2007).

common food sources of choline and betaine may differ from Caucasians: e.g., more choline may be obtained from plant-based foods, such as soybean products among Asian Americans and beans among Latinos (21), which have a different composition of choline esters compared with the composition in animal-based foods. Therefore, in the current study, we tested the feasibility of using the publicly available reference FCDs to assess the dietary intake of choline, choline-containing compounds, and betaine among the participants of a large multi-ethnic cohort study and examined the variation in the intake estimates by sex and race/ethnicity.

## Participants and Methods

**Study population: the multiethnic cohort.** We estimated the dietary intake of choline and betaine among the participants of the Multiethnic Cohort (MEC), a prospective follow-up study established in 1993–1996. The MEC includes >215,000 men and women aged 45–75 y at recruitment, primarily from 5 ethnic/racial groups residing in Hawaii (mostly Caucasians, Japanese Americans, and Native Hawaiians) and Los Angeles, California (mostly African Americans and Latinos) (22). Potential participants were identified from the Hawaii and Los Angeles resident population using drivers' license files, the Health Care Financing Administration files, and the Hawaii voters' registration file. Participants gave consent and participated in the cohort by mailing back a self-administered, 26-page questionnaire, which included a quantitative FFQ for the habitual dietary intake during the previous year as well as queries on demographics and various risk factors for cancers (22). Response rates varied from 20% in Latinos to 49% in Japanese Americans, yielding a broadly representative group, as evidenced by the cohort distributions across education levels and marital status similar to corresponding census data for the areas (22). The Institutional Review Boards at the University of Hawaii and the University of Southern California approved the study.

**Updating the FCD for the MEC FFQ.** The MEC quantitative FFQ includes direct inquiries for >180 line-item foods (i.e., single or multiple similar foods in each of >180 questions). The FFQ was developed from 3-d food records collected on a representative sample of the 5 main ethnic groups and was designed to capture the majority (>85%) of ethnic-specific nutrient intake in the MEC (22). The FFQ was validated based on satisfactory correlations with three 24-h dietary recalls (23).

A specific FCD has been developed for the MEC FFQ. It requires food composition information on ~1000 single foods to calculate a nutrient profile for the items on the FFQ. We updated the MEC FFQ-specific FCD with choline, choline-containing compound (or choline compound), and betaine content using 2 publicly available references. The latest USDA Database for the Choline Content of Common Foods (Release Two, 2008, heretofore referred to as the "choline database") (17) contains information on total and component choline and betaine for 634 food items in 22 categories and was the preferred source for obtaining values. The USDA National Nutrient Database for Standard Reference (Release 20, 2007) (SR) (19) has information on only total choline and betaine content and covered additional food items ( $n = 3299$  and 1214 items for total choline and betaine, respectively). Also, a few values were obtained from the published work by Zeisel et al. (24).

When exact matches to one of these databases were not possible, values were imputed. Decisions on imputations were made case by case and following a systematic decision algorithm (Supplemental Fig. 1). The imputation decisions and results were confirmed or modified in further group discussion and were independently spot-checked.

**Assignment of total choline values.** Food items needed to calculate the MEC FFQ-specific FCD were matched to the reference FCDs by the unique nutrient databank number using an automated process, first to the choline database and, if no match was found, then to the SR database. Total choline values for foods without a direct match in the reference FCDs were imputed, first based on the same food with a different description (e.g., total choline value of ready-to-eat chocolate

pudding in SR assigned to chocolate pudding in MEC FCD). If a close match by description could not be found, a mean of the choline values available from the same food in different forms (e.g., cooked and raw versions or different parts of the same food), from similar foods (e.g., cooked green leafy vegetables for cooked tree fern), or from the same food category (e.g., winter and summer squash for sequa; different spices for allspice) was assigned. Nutrient retention factors (per 100 g food) from the USDA (Release 6) (25) were used to estimate the total choline value of a cooked food item based on the reference value available for a raw item ( $n = 49$ ). For foods with multiple ingredients (e.g., French toast with egg, sugar, milk, bread, and shortening), total choline values were assigned by calculating the percent weight contribution of each ingredient multiplied by its choline content per 100 g and then summing over all ingredients. Certain plant food items in the botanical family or genus with an overall low choline contribution were assigned the values of another member with most similar appearance and texture (e.g., green beans for nopales). After the imputation process, one item remained [monosodium glutamate (MSG)] and was assigned a zero value for total choline, because this additive does not contain a choline moiety.

**Assignment of choline-containing compound values.** Choline-containing compound values were available only in the choline database and not from the SR. For the MEC FCD food items without a matching reference item, choline compound values were assigned by multiplying the assigned total choline value by the ratio of choline compounds found in the closest item, while identifying the closest item following the same imputation algorithm used for total choline value assignments (Supplemental Fig. 1). For example, the choline compound values for gai lan (Chinese broccoli), which was assigned a total choline value directly from the SR, were estimated based on the mean of 2 similar items with different levels of total choline (broccoli with 40 mg/100 g and kale with 0.4 mg/100 g) by multiplying the total choline value for gai lan (25.3 mg/100 g from the SR) by the mean ratio of choline compounds in broccoli [free choline (21%):glycerophosphocholine (3%): phosphocholine (23%):phosphatidylcholine (53%):sphingomyelin (0%) from the choline database] and kale (25:0:0:75:0% from the choline database). In another example, the choline compound values for hasu (lotus root) were calculated based on the mean choline compound ratio from the ratios of all other root vegetables, such as carrots and radishes. As with total choline, choline compounds were assumed to be zero for MSG.

**Assignment of betaine values.** Assignment of betaine values to the MEC FCD food items was conducted using a process similar to that for total choline. The 8 remaining food items [bamboo shoots (canned, drained), salt, vinegar, water, saccharin, aspartame, corn syrup, and MSG] were reasonably assumed to have zero betaine values based on the known nutrient composition of these and related items [e.g., near-zero betaine content in celery, cucumber, ginger root, onions, gobo (burdock root), etc., justified a zero value for bamboo shoots].

**Estimation and statistical analysis of individual dietary choline and betaine intake among MEC participants.** Daily intake of total choline, choline-containing compounds, and betaine among the MEC participants at cohort baseline was estimated by multiplying the nutrient content for each FFQ item in the reported serving size (small, medium, large) by the consumption frequency (8 or 9 categories, from "never or hardly ever" to "2 or more times a day") and by summing up the nutrient intake amount for all food items for each individual. Individuals who belonged to other ethnic groups than the 5 primary groups of interest ( $n = 13,994$ ) or who reported extreme energy and/or macronutrient intakes that were likely to be errors ( $n = 8265$ ) were excluded from the analysis (26). We further excluded individuals ( $n = 5425$ ) who did not report their body weight, height, or smoking status (never, former, current) or who had extreme BMI values ( $<15$  or  $>70$  kg/m<sup>2</sup>), as these individuals are routinely excluded in the MEC analyses of diet/lifestyle and disease associations to prevent spurious findings (27). For the remaining 188,147 participants, the mean intake value for each nutrient (total choline, choline-containing compounds, betaine) was compared between men and women and across the 5 ethnic groups within each sex in general linear models, where the overall ethnic heterogeneity was determined

by *P*-heterogeneity for the *Fisher* statistic and the difference of each non-Caucasian group from Caucasians by *P*-difference for the pairwise comparison. Means were obtained with and without adjustment for total energy intake, and sex-specific means were further adjusted for ethnic composition. Analyses were conducted using SAS v9.3 and differences were considered significant at *P* < 0.05 (2-sided), which allows detection of small effect sizes (*d*) with our large sample size (*d* = 0.04 between the smallest ethnic groups and *d* = 0.02 between sexes) (28). Primary food group sources of choline and betaine consumed by the MEC participants were determined using the MyPyramid Equivalents Database food subgroups (29), with a modification to combine fish and shellfish with high and low contents of omega-3 fatty acids, because this classification appears to be irrelevant to the content of choline and betaine.

## Results

Following the algorithm in Supplemental Figure 1, we assigned total choline, choline compound, and betaine values to the 965 single food items required for the intake estimation using the MEC FFQ. As shown in Table 1 and summarized in Supplemental Figure 2, 736 (76%) items were assigned total choline values by direct match based on the nutrient databank number; 124 items (13%) were imputed from the same food item with different description, status, or parts, 98 items (10%) were imputed from similar food items, and 6 items (1%) were imputed from the same food category. Compared with total choline values, choline compound and betaine values were assigned from fewer direct matches (*n* = 189 and 337, respectively) and were imputed more from the same foods (*n* = 300, 236), similar foods (*n* = 284, 227), or same food categories (*n* = 191, 157). Only a few items remained (*n* = 1, 1, and 8 for total choline, choline compounds, and betaine, respectively) that could be reasonably assumed to have zero values as described in the Methods.

The demographic characteristics of the MEC were previously reported in detail (22). Choline and betaine intake estimates for the MEC participants at baseline are presented in Table 2 by sex and race/ethnicity. Men consumed significantly higher amounts of total choline, choline-containing compounds, and betaine

than women (*P*-heterogeneity for sex < 0.0001), except for phosphocholine (*P*-heterogeneity = 0.12). The mean choline and betaine intake estimates among men and women of the MEC were within the range of intakes reported in 3 other cohorts, with generally greater variations observed in the MEC (Supplemental Table 1) (13). When the nutrients were adjusted for daily energy intake to compare the nutrient density at a given level of energy consumption, men and women had much more similar levels of adjusted total choline (337 vs. 333 mg/d; *P* < 0.0001) and betaine intake (140 vs. 139 mg/d; *P* = 0.20).

In both men and women, intake estimates varied significantly by race/ethnicity for choline and betaine (overall *P*-heterogeneity for ethnicity < 0.0001), with a similar variation shown for the proportion of individuals who consumed less than the AI amount of choline (Table 2). In pairwise comparisons with Caucasians (372 mg/d in men, 306 mg/d in women), energy-adjusted choline intake was higher among African Americans (381 mg/d in men, 312 mg/d in women) and Latinos (390 and 314 mg/d, respectively) and lower among Japanese Americans (356 and 289 mg/d, respectively) and Native Hawaiians (366 and 297 mg/d, respectively) (Fig. 1). Betaine intake also varied among the ethnic groups (all *P*-differences between non-Caucasians and Caucasians < 0.0001). Energy-adjusted betaine intake was similar or lower in all sex/ethnic groups compared with their Caucasian counterparts (168 mg/d in men, 136 mg/d in women) (Fig. 1).

Table 3 shows the primary food sources of choline and betaine among MEC participants. Red meats contributed the most to total choline intake in all ethnic groups, except in African Americans, who obtained more choline from poultry (data not shown). Animal-based foods with high choline content (meats, eggs, poultry, and milk) provided 40.9% of total choline intake, followed by non-whole grains and vegetables other than dark-green, orange, or starchy types and tomatoes. Ethnic-specific choline sources ranked fish and shellfish higher among Japanese Americans and Native Hawaiians (5.6% in both groups) and beans and peas higher among Latinos (7.8%; data not shown) than in other ethnic groups. The majority of betaine intake was

**TABLE 1** Assignment of choline and betaine content to food items in the food composition database for the MEC FFQ<sup>1</sup>

Matching or imputation method	Food items, <i>n</i> (%)		
	Total choline	Choline-containing compounds	Betaine
(1) Direct match to the USDA Choline Database	189 (19.6)	189 (19.6)	189 (19.6)
(2) Direct match to the USDA SR Database	547 (56.6)	0 (0)	148 (15.3)
(3a) Imputed from same food item/part, same status	61 (6.3)	78 (8)	41 (4.3)
(3b) Imputed from same food item/part, different status	27 (2.8)	126 (13.1)	117 (12.1)
(3c) Imputed from same food item/part, different status, with retention value	20 (2.1)	1 (0.1)	4 (0.4)
(3d) Imputed from same food item, different part, same status	9 (0.9)	25 (2.6)	30 (3.1)
(3e) Imputed from same food item, different part, different status	2 (0.2)	54 (5.6)	8 (0.8)
(3f) Imputed from same food item, different part, different status, with retention value	0 (0)	1 (0.1)	0 (0)
(3g) Imputed from same food, calculated (for mixed foods)	5 (0.5)	15 (1.6)	36 (3.7)
(4a) Imputed from similar food item, same status	70 (7.3)	219 (22.7)	196 (20.3)
(4b) Imputed from similar food item, different status	28 (2.9)	65 (6.7)	31 (3.2)
(5) Imputed from same food category	6 (0.6)	191 (19.8)	157 (16.3)
(6) Assumed zero value	1 (0.1)	1 (0.1)	8 (0.8)
(7) Cannot match or impute	0	0	0
Total	965	965	965

<sup>1</sup> Choline database, USDA Database for the Choline Content of Common Foods (Release Two, 2008); MEC, Multiethnic Cohort; SR, USDA National Database for Standard Reference (Release 20, 2007).

**TABLE 2** Dietary choline and betaine intakes in the MEC at baseline (1993–1996) by sex and race/ethnicity<sup>1</sup>

	<i>n</i>	Total choline	<AI	Free choline	Glycero-phosphocholine	Phospho-choline	Phosphatidylcholine (lecithin)	Sphingomyelin	Betaine
		mg/d	%				mg/d		
Men	85,784	372 ± 187	86	93 ± 56	61 ± 37	14 ± 8	184 ± 104	20 ± 12	154 ± 96
Caucasian	21,799	359 ± 151	89	93 ± 49	63 ± 35	15 ± 7	169 ± 82	19 ± 9	163 ± 90
African American	11,673	354 ± 192	86	81 ± 52	55 ± 37	14 ± 8	184 ± 109	21 ± 13	151 ± 98
Japanese American	25,754	335 ± 143	92	85 ± 46	53 ± 29	12 ± 6	166 ± 80	17 ± 9	147 ± 85
Latino	20,578	427 ± 234	77	106 ± 67	68 ± 44	17 ± 10	213 ± 129	23 ± 14	147 ± 103
Native Hawaiian	5980	425 ± 225	78	103 ± 69	70 ± 46	16 ± 10	214 ± 123	23 ± 13	181 ± 118
<i>P</i> -heterogeneity <sup>2</sup>		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>P</i> -heterogeneity <sup>3</sup>		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Women	102,363	304 ± 153	84	75 ± 39	49 ± 28	14 ± 8	149 ± 85	16 ± 10	128 ± 80
Caucasian	25,177	287 ± 115	88	74 ± 32	51 ± 26	15 ± 7	132 ± 62	15 ± 7	128 ± 73
African American	20,013	304 ± 161	82	70 ± 40	46 ± 29	14 ± 8	155 ± 90	18 ± 11	132 ± 85
Japanese American	28,097	268 ± 110	91	67 ± 28	42 ± 21	12 ± 6	132 ± 61	13 ± 7	121 ± 70
Latino	21,445	350 ± 193	74	86 ± 47	55 ± 33	17 ± 10	173 ± 108	19 ± 12	122 ± 85
Native Hawaiian	7631	358 ± 198	73	86 ± 51	58 ± 36	16 ± 11	179 ± 108	19 ± 12	159 ± 105
<i>P</i> -heterogeneity <sup>2</sup>		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>P</i> -heterogeneity <sup>3</sup>		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>P</i> -heterogeneity <sup>4</sup>		<0.0001	<0.0001	<0.0001	<0.0001	0.12	<0.0001	<0.0001	<0.0001
<i>P</i> -heterogeneity <sup>5</sup>		<0.0001	<0.0001	<0.0001	<0.0001	0.12	<0.0001	<0.0001	0.20

<sup>1</sup> Values are means ± SDs other than those specified as *n* or % below AI. AI, adequate intake level (550 mg/d for men, 425 mg/d for women); MEC, Multiethnic Cohort.

<sup>2</sup> *P* value for heterogeneity by ethnicity in unadjusted general linear model.

<sup>3</sup> *P* value for heterogeneity by ethnicity in general linear model adjusted for total energy intake.

<sup>4</sup> *P* value for heterogeneity by sex in unadjusted general linear model.

<sup>5</sup> *P* value for heterogeneity by sex in general linear model adjusted for ethnicity and total energy intake.

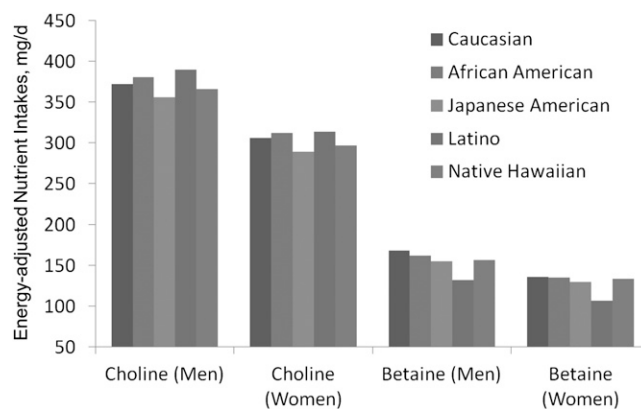
from grains (58.4%), followed by dark-green vegetables (12.6%) and alcohol (7.3%; mostly attributed to beer) and was similar across ethnic groups. The top non-whole-grain item in the MEC FFQ for betaine consumption was pasta (line item of “spaghetti, ravioli, lasagna, or other pasta”; among combined ethnicities, whites and African Americans), saimin/ramen (Oriental noodles with broth; among Japanese Americans and Native Hawaiians), or fortified cereals (among Latinos), followed by white bread. The 10 primary food sources of total choline and betaine contributed 68 and 88% of total intake, respectively.

## Discussion

We estimated dietary choline and betaine intakes among ethnically diverse adults using publicly available food composition references and found that consumption varied by ethnicity, even after adjusting for energy intake differences, although some of these differences were very small. Assuming accurate USDA reference data, our total choline intake estimation should be reliable, because 76% of the ~1000 food items used in our study-specific dietary assessment tool were directly matched to the reference items and another 13% were imputed from the same food in the references. Fewer betaine values were based on direct matches (35%) or same food-based imputations (24%), but the majority of betaine consumption in our study population, as in others, was from non-whole- and whole-grain items, for which the reference FCDs are detailed.

Our overall and Caucasian-specific estimates for total choline and choline compound (free choline and choline esters) intake in MEC were generally comparable with the estimates reported for the mostly Caucasian adult participants in the Framingham Offspring Study (18), the Nurses’ Health Study (13), and the Atherosclerosis Risk in the Communities Study (15). On the

other hand, the mean betaine intake in MEC based on the second version of the USDA Database for the Choline Content of Common Foods was generally lower than the estimates in the previous studies that used the first version of the database. This was expected, because the betaine content of some primary sources (grain products, such as cereal, bread, and pasta, as well as spinach and seafood) has been corrected to substantially lower values in the later version (17).



**FIGURE 1** Energy-adjusted choline and betaine intakes in the MEC at baseline (1993–1996) by sex and race/ethnicity. Values are means, *n* = 85,784 (men) and 102,363 (women). Difference between men and women for choline (*P* < 0.0001), but not for betaine (*P* = 0.20), difference across ethnicities in men (*P* < 0.0001) and women (*P* < 0.0001), and difference between each non-Caucasian group vs. Caucasians in men (choline *P* < 0.0001 for African Americans, Japanese Americans and Latinos, *P* = 0.0004 for Native Hawaiians; betaine *P* < 0.0001 for all groups) and women (choline *P* < 0.0001 for all groups; betaine *P* < 0.0001 for all groups). MEC, Multiethnic Cohort.

**TABLE 3** Primary food groups contributing to choline and betaine intakes in the MEC at baseline (1993–1996)<sup>1</sup>

Rank	Total choline		Betaine	
	Food sources <sup>2</sup>	Contribution, %	Food sources <sup>2</sup>	Contribution, %
1	Meats	13.1	Non-whole grains	43.1
2	Eggs	10.4	Whole grains	15.3
3	Poultry	9.9	Dark-green vegetables	12.6
4	Milk	7.5	Alcohol	7.3
5	Non-whole grains	7.2	Meats	2.9
6	Other vegetables	4.5	Poultry	1.9
7	Fish, shellfish	3.9	Fish, shellfish	1.4
8	Alcohol	3.9	Milk	1.3
9	Other fruits	3.8	Soybean products	1.1
10	Beans, peas	3.6	Other starchy vegetables	1.0
Sum		67.8		87.9

<sup>1</sup> MEC, Multiethnic Cohort.

<sup>2</sup> Food source categories are based on the MyPyramid Equivalents Database food subgroups: alcohol (beer, wine, hard liquor, other alcohol); meats (beef, veal, lamb, pork, ham, bacon); milk (whole, 2%, low-fat, skim, and ice milk); orange vegetables (carrots, pumpkin, other yellow vegetables, carrot juice); other fruits (fruits other than citrus fruits, melons, and berries; including red papaya, other pink fruits, mango, yellow papaya, peaches, apricots, other yellow/orange fruit, apple, banana, pear, pineapple, green papaya, green mango, other miscellaneous fruits, other fruit juice); other vegetables (vegetables excluding dark-green, orange, and starchy vegetables and tomatoes; light-green cruciferous, asparagus, light-green lettuce, other light-green vegetables, cauliflower, other cruciferous, onion, seaweed, other miscellaneous vegetables); other starchy vegetables (corn and tubers, other than potatoes); soybean products (tofu, soy other than tofu, soy sauce).

Differences in choline and betaine intake across ethnic groups, as well as between men and women, in the MEC were in part due to varying energy intakes. Mean energy-adjusted choline and betaine intakes significantly differed by sex and across ethnic groups, in part due to the large sample size of the study. For example, the difference in choline intake between men (337 mg/d) and women (333 mg/d;  $P < 0.0001$ ) was small but highly significant. However, the physiological importance of these differences is not clear, even for the larger ethnic differences that were seen for total choline (difference in energy-adjusted means comparing Caucasians and Japanese Americans of up to  $-16$  and  $-17$  mg/d for men and women, respectively) and betaine (up to  $-36$  and  $-29$  mg/d for men and women) intakes.

With or without adjustment for energy intake, nearly three-quarters or more of all sex/ethnic groups had choline intakes below the AI level, a finding that is consistent with those of other studies. However, this result needs to be interpreted with caution, because the prevalence of inadequate intakes for nutrients with an AI cannot be estimated. The AI for choline was estimated based on an experiment where an intake of  $\sim 7$  mg/(kg · d) choline normalized the blood liver enzyme levels in 8 healthy young men after a choline-depletion diet (11,12). Although a choline-deficient diet, with or without methionine deficiency, is known to induce nonalcoholic fatty liver disease and other liver damage (30), the mechanism does not appear to involve insulin resistance, unlike in most obesity-associated fatty liver cases (31). Thus, the ethnic differences in choline intake (higher among Latinos and lower among Japanese Americans compared with Caucasians in our study) may not explain the ethnic variation in nonalcoholic fatty liver disease, which is more common among Latinos and Asians and less common among African Americans compared with Caucasians (32,33). More research is needed to determine dietary choline requirements (12). In particular, the improved folate nutrition of the U.S. population due to the nationwide folic acid fortification of grains may reduce choline requirements, as *de novo* synthesis of choline depends on the folate supply (34).

Energy-adjusted betaine intake was particularly low among Latinos, likely due to low betaine content in the staples they consume, such as rice and tortillas. These staples, as well as potatoes, provide minimal amounts of betaine ( $<3$  mg in 2 corn or flour tortillas;  $<1$  mg in 87 g of cooked rice or one-half of a medium-sized, baked white potato), whereas other wheat-based staples, such as pasta ( $\sim 25$  mg in 114 g, cooked), white bread ( $\sim 16$  mg in 2 slices), or breakfast cereal ( $\sim 10$  mg in 60 g), contain higher amounts for a similar amount of weight or energy.

This study has some limitations that need consideration. As with other nutrient analyses, the accuracy of choline and betaine intake estimation is limited by the variability in both natural and processed foods as well as in laboratory analyses of food samples (17). Currently, an indicator of the nutrient data quality is provided by the USDA for selected nutrients (the quality index, which ranges from 1 to 100%) and is based on an evaluation of the food item sampling plan, sample handling, number of samples analyzed, analytical method, and analytical quality control. A quality index is available for total choline data (but not for betaine or choline-containing compounds), with the majority of the food items (79%) in the reference databases scoring in the range of 50–74% (17). However, choline-containing compounds were estimated with less certainty than total choline, due to the limited availability of reference values, and would need future validation and expansion to include more ethnic food items.

Another limitation of this and most large epidemiologic studies is that the dietary assessment was based on a one-time self-report of a typical diet consumed in the near past. One previous study indicated that the intra-individual variability for reported choline and betaine consumption was similar to that observed for other nutrients (all reliability coefficients at  $\sim 0.50$ ) when a subgroup of individuals completed the same FFQ 3 y apart (15,35). Also, FFQs have a limited capacity to estimate absolute intake amounts and perform better when ranking people for their relative intake (36). Thus, our estimation of individual intake and percent individuals with intake below the recommended level should be interpreted with caution. Our

study also did not include a biomarker validation of the dietary assessment. However, Cho et al. (18) previously observed a strong inverse association between blood concentrations of homocysteine, a commonly used biomarker of 1-carbon metabolism, and dietary intake of choline or betaine.

The strengths of this study are that intake of several under-studied nutrients were estimated in a large population-based cohort with a broadly representative sample of 5 ethnic groups and using a dietary instrument developed to capture most of the ethnic variations. This work will be updated in the future as more information on the choline and betaine content of foods becomes available. These intake estimates will provide the basis for unique examinations of the health impact of choline and betaine nutrition in our ethnically diverse populations.

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